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This invention provides a sustained-release preparation production method comprising production of a W/O emulsion whose internal aqueous phase is a solution containing a bioactive peptide represented by formula (I), wherein X represents a hydrogen atom or a tetrahydrofurylcarboxamido; Q represents a hydrogen atom or methyl; A represents nicotinoyl or N,N'-diethylamidino; B represents isopropyl or N,N'-diethylamidino, or a salt thereof, and whose oil phase is a solution containing a biodegradable polymer having a free carboxyl group at one end, and adding the W/O emulsion into an external water phase to produce W/O/W emulsion. According to the production method of the present invention, a sustained-release preparation containing peptide (I) or a salt thereof can be obtained easily and at high recovery rates.

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## DESCRIPTION

## SUSTAINED-RELEASE PREPARATION AND USE

5     Technical Field

The present invention relates to a method of producing a sustained-release preparation containing a bioactive peptide possessing LH-RH-antagonizing activity or a salt thereof.

10     Background Art

As a prior art method, EP-A-601,799, for instance, describes a method of producing a sustained-release preparation (in-water drying method using an O/W emulsion, phase separation method and spray drying method), by  
15     dissolving both a bioactive peptide and a biodegradable polymer having a free carboxyl group at one end in a substantially water-immiscible solvent, then removing the solvent.

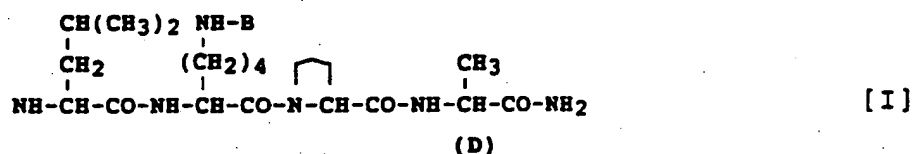
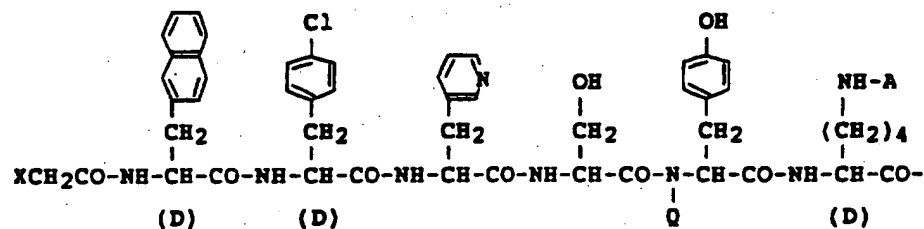
20     Disclosure of Invention

Although use of first- or second-generation LH-RH (lutein-izing hormone-releasing hormone) antagonists has been problematic because of their histamine-releasing  
25     action (Gekkan Yakuji, Vol. 32, pp. 1599-1605, 1990), a large number of compounds have been synthesized, resulting in the recent development of LH-RH-antagonizing bioactive peptides without the problem of histamine-releasing action (e.g., Japanese Patent Unexamined Publication No.  
30     101695/1991). For such LH-RH-antagonizing bioactive peptides to exhibit pharmaceutical effect, they must competitively inhibit LH-RH action constantly in the body. Accordingly, there is need for the development of sustained-release preparations such peptides. In addition,  
35     there is also need for the development of a method of producing a sustained-release preparation in which excess

drug release is suppressed just after administration, since such bioactive peptides possess low but not negligible histamine-releasing activity. Also, in sustained-release preparations of the long-acting type (e.g., 1-3 months), more reliable, constant release of bioactive peptide is a key to safe and more reliable effect. There is need for a method of producing a sustained-release preparation that constantly releases a bioactive peptide and that possesses excellent storage stability.

The present invention relates to:

(1) a method of producing a sustained-release preparation, which comprises producing a W/O emulsion whose internal aqueous phase is a solution containing a bioactive peptide represented by the formula:



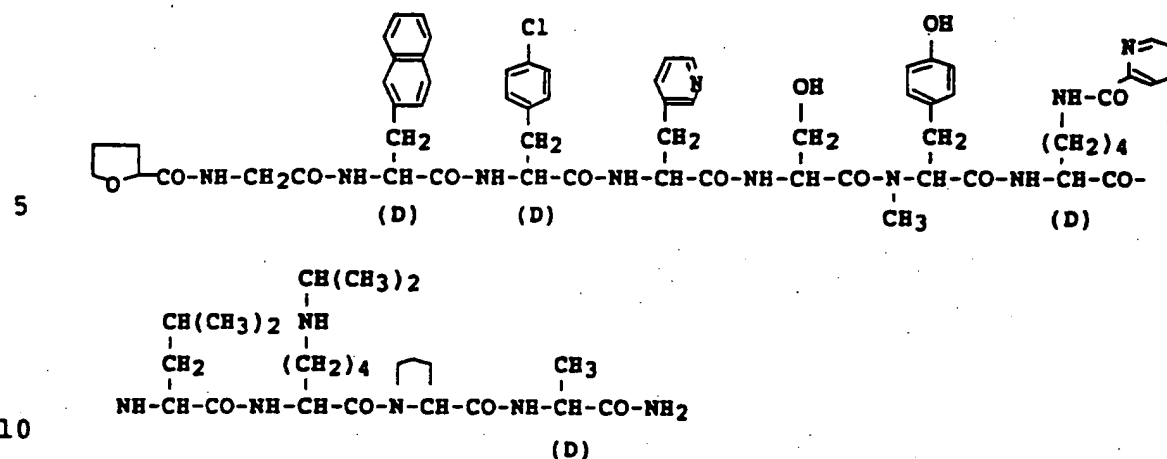
wherein X represents a hydrogen atom or a tetrahydrofuryl-carboxamido; Q represents a hydrogen atom or methyl; A represents nicotinoyl or N,N'-diethylamidino; B represents isopropyl or N,N'-diethylamidino, or a salt thereof, and whose oil phase is a solution containing a biodegradable polymer having a free carboxyl group at one end, and adding the W/O emulsion into an external water phase to produce W/O/W emulsion,

(2) a method of term 1 above, wherein the biodegradable polymer is an aliphatic polyester,

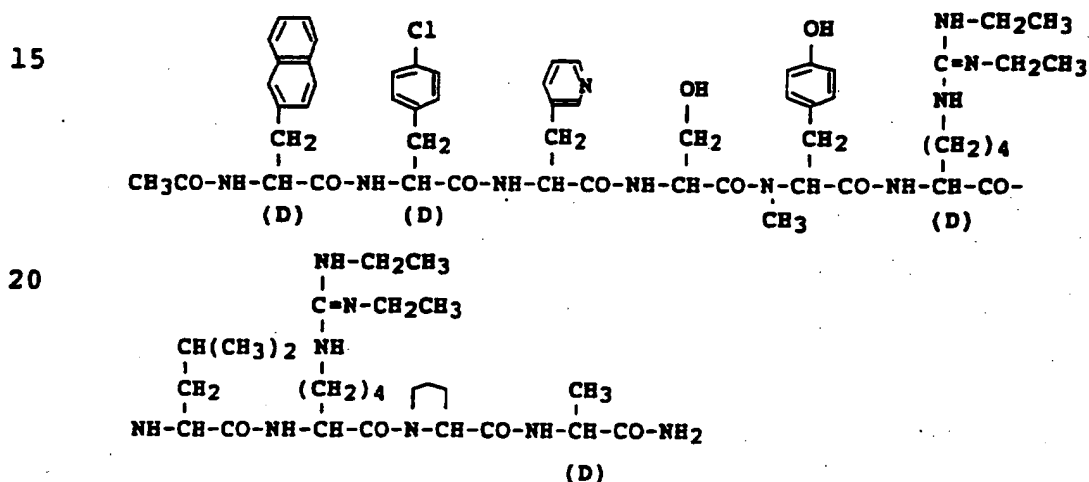
- (3) a method of term 2 above, wherein the aliphatic polyester is a lactic acid-glycolic acid copolymer,  
(4) a method of term 3 above, wherein a composition ratio of lactic acid and glycolic acid is about 100/0 to about 40/60 (mole%),  
5 (5) a method of term 3 above, wherein a weight-average molecular weight of the copolymer is about 5,000 to about 25,000,  
(6) a method of term 1 above, wherein a peptide  
10 concentration in the internal aqueous phase is about 0.1 to about 150% (w/v),  
(7) a method of term 1 above, wherein a polymer concentration in the oil phase is about 0.01 to about 80% (w/w),  
15 (8) a method of term 1 above, wherein a volume ratio of the internal aqueous and oil phase is about 1 to about 50% (v/v),  
(9) a method of term 1 above, wherein a volume of the external aqueous phase is about 1 to about 10,000 times that of the oil phase,  
20 (10) a method of term 1 above, wherein the preparation is microcapsules,  
(11) a method of term 1 above, wherein X is 2-tetrahydrofurylcarboxamido,  
25 (12) a method of term 11, wherein the 2-tetrahydrofurylcarboxamido is (2S)-tetrahydrofurylcarboxamido,  
(13) a method of term 1 above, wherein the peptide is of the formula:

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35



(14) a method of term 1 above, wherein the peptide is of the formula:



(15) a sustained-release preparation, which is produced by the method of term 1 above,

(16) a preparation of term 15 above, wherein a content ratio of the peptide is about 0.01 to about 50% (w/w), relative to the polymer,

(17) a preparation of term 15 above, wherein the preparation is microcapsules, and

(18) a preparation of term 17 above, wherein the microcapsules are for injection.

Abbreviations used in the present specification have the following meanings.

NACD2Nal : N-acetyl-D-3-(2-naphthyl)alanyl  
D4ClPhe : D-3-(4-chlorophenyl)alanyl  
D3Pal : D-3-(3-pyridyl)alanyl  
NMeTyr : N-methyltyrosyl  
5 DLys(Nic) : D-(ipsiron-N-nicotinoyl)lysyl  
Lys(Nisp) : (Ipsiron-N-isopropyl)lysyl  
DhArg(Et<sub>2</sub>) : D-(N,N'-diethyl)homoarginyl

Abbreviations for other amino acids are based on abbreviations specified by the IUPAC-IUB Commission on Biochemical Nomenclature (European Journal of Biochemistry, 10 Vol. 138, pp. 9-37, 1984) or abbreviations in common use in relevant fields. When an optical isomer may be present in amino acid, it is of the L-configuration, unless otherwise stated.

15 In the present invention, the bioactive peptide represented by formula [I] (hereinafter also referred to as peptide [I]) or a salt thereof possesses LH-RH-antagonizing activity, and accordingly, is effective in the treatment of  
20 hormone-dependent diseases, such as prostatic cancer, prostatic hypertrophy, endometriosis, uterine myoma, uterine fibroma, precocious puberty, breast cancer, bladder cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, gastritis,  
25 hodgekin's disease, malignant melanoma, metastasis, multiple myeloma, non-hodgekin's leukemia, non-small-cell lung cancer, ovarian cancer, peptic ulcer, serious fungal infection, small-cell lung cancer, valvular heart disease, mastopathy, polycystic ovary, infertility, controlled  
30 induction of ovulation in women with chronic anovulation, acne, amenorrhea (e.g., secondary amenorrhea), ovarian and mammary cystic disease (including, polycystic ovarian diseases), gynecological cancer, ovarian hyperandrogenism and hirsutism, AIDS by rejuvenating the thymus to produce  
35 T-cells, male contraceptives for the treatment of male sex

offenders and in contraception, symptomatic relief of the premenstrual syndrome (PMS), in vitro fertilization.

5 With respect to the formula [I], X is preferably 2-tetrahydrofurylcarboxamido, more preferably (2S)-tetrahydrofurylcarboxamido. Also, A is preferably nicotinoyl; B is preferably isopropyl.

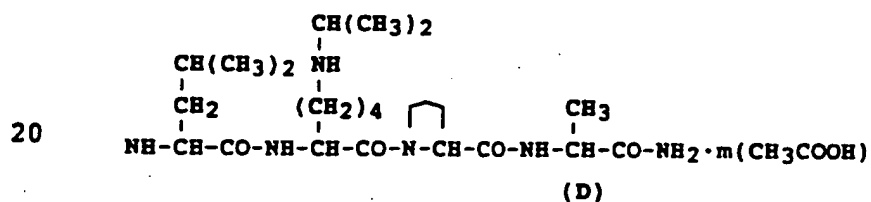
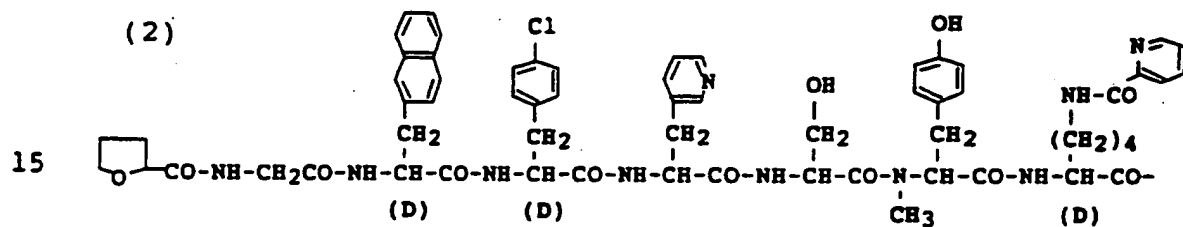
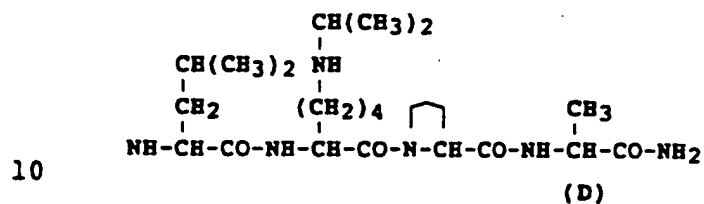
10 When peptide [I] has one or more kinds of asymmetric carbon atoms, two or more optical isomers are present. Such optical isomers and mixtures thereof are also included in the scope of the present invention.

15 Peptide [I] or a salt thereof can be produced by known methods, which include those methods described in Japanese Patent Unexamined Publication No. 101695/1994 and the Journal of Medicinal Chemistry, Vol. 35, p. 3942 (1992) and other publications, and similar methods.

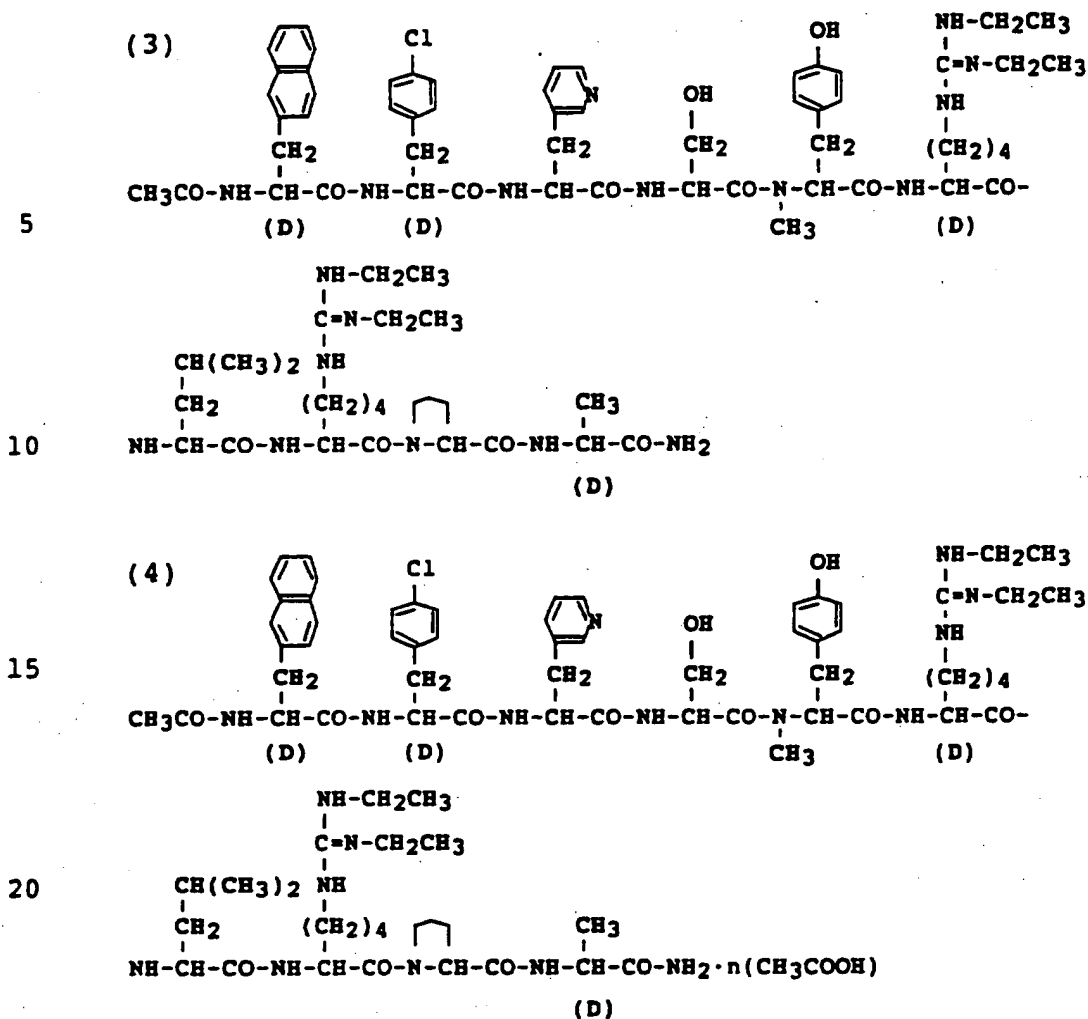
20 The salt of peptide [I] is preferably a pharmacologically acceptable salt. Such salts include salts with inorganic acids (e.g., hydrochloric acid, sulfuric acid, nitric acid, etc.), organic acids (e.g., carbonic acid, bicar-bonic acid, succinic acid, acetic acid, propionic acid, trifluoroacetic acid, etc.) etc. More preferably, the salt of peptide [I] is a salt with an organic acid  
25 (e.g., carbonic acid, bicarbonic acid, succinic acid, acetic acid, propionic acid, trifluoroacetic acid, etc.), with greater preference given to the salt with acetic acid. These salts may be mono- to tri-salts, with preference given to di- or tri-salts.

30 Examples of particularly preferable peptide [I] compounds or salts thereof are given below.

35



wherein, m represents an integer from 1 to 3.



wherein, n represents an integer from 1 to 3.

25 Peptide [I] or salt thereof is preferably (1) or (2) above.

30 The biodegradable polymer having a free carboxyl group at one end is a biodegradable polymer whose GPC measurement- and terminal group quantitation-based number-average molecular weights almost agree with each other.

Number-average molecular weight based on terminal group quantitation is calculated as follows:

35 About 1 to 3 g of the biodegradable polymer is dissolved in a mixed solvent of acetone (25 ml) and

methanol (5 ml); the solution is quickly titrated with a 0.05 N alcoholic solution of potassium hydroxide while stirring at room temperature (20°C), with phenolphthalein as an indicator to determine carboxyl group content; the number-average molecular weight is calculated from the following equation:

Number-average molecular weight based on terminal group quantitation =  $20,000 \times A/B$

where A is the weight mass (g) of the biodegradable polymer, and B is the amount (ml) of the 0.05 N alcoholic solution of potassium hydroxide added until the titration end point is reached.

For example, in the case of a polymer having a free carboxyl group at one end, and synthesized from one or more  $\alpha$ -hydroxy acids by catalyst-free dehydration polymerization condensation, the GPC measurement- and terminal group quantitation-based number-average molecular weights almost agree with each other. On the other hand, in the case of a polymer having substantially no free carboxyl group at one end, and synthesized from a cyclic dimer by ring-opening polymerization using a catalyst, the number-average molecular weight based on terminal group quantitation is significantly higher than that based on GPC measurement. This difference makes it possible to clearly differentiate a polymer having a free carboxyl group at one end from a polymer having substantially no free carboxyl group at one end.

While the number-average molecular weight based on terminal group quantitation is an absolute value, that based on GPC measurement is a relative value, that varies depending on various analytical conditions (e.g., kind of mobile phase, kind of column, reference substance, slice width, baseline). It is therefore difficult to have an absolute numerical representation of both values. However, the fact that the GPC measurement- and terminal group

quantitation-based number-average molecular weights almost agree with each other means that the number-average molecular weight based on terminal group quantitation falls within the range from about 0.4 to about 2 times, preferably from about 0.5 to about 2 times, and more preferably from about 0.8 to about 1.5 times, that based on GPC measurement. Also, the fact that the number-average molecular weight based on terminal group quantitation is significantly higher than that based on GPC measurement means that the number-average molecular weight based on terminal group quantitation is about 2 times or more that based on GPC measurement.

Examples of biodegradable polymers having a free carboxyl group at one end include polymers, copolymers, or mixtures thereof, synthesized by catalyst-free dehydration polymerization condensation from one or more  $\alpha$ -hydroxycarboxylic acids (e.g., glycolic acid, lactic acid, hydroxybutyric acid, etc.), hydroxydicarboxylic acids (e.g., malic acid, etc.), hydroxytricarboxylic acids (e.g., citric acid, etc.) etc., poly- $\alpha$ -cyanoacrylates, polyamino acids (e.g., poly- $\gamma$ -benzyl-L-glutamic acid, etc.), maleic anhydride copolymers (e.g., styrene-maleic acid copolymer, etc.) and the like.

The biodegradable polymer is preferably an aliphatic polyester such as a homopolymer, copolymer or mixture thereof synthesized from one or more  $\alpha$ -hydroxycarboxylic acids (e.g., glycolic acid, lactic acid, hydroxybutyric acid, etc.), hydroxydicarboxylic acids (e.g., malic acid, etc.), hydroxytricarboxylic acids (e.g., citric acid, etc.) and so on.

Polymerization may be of the random, block or graft type. When the above-mentioned  $\alpha$ -hydroxy acids, hydroxydicarboxylic acids and hydroxytricarboxylic acids have an optically active center in their molecular structures, they may be of the D-, L- or DL-configuration.

The biodegradable polymer having a free carboxyl group at one end is preferably (1) a lactic acid-glycolic acid copolymer or (2) a biodegradable polymer comprising a mixture of (A) a copolymer of glycolic acid and a hydroxy-carboxylic acid represented by the formula:



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wherein R represents an alkyl group having 2 to 8 carbon atoms, and (B) polylactic acid. More preferably, the biodegradable polymer having a free carboxyl group at one end is a lactic acid-glycolic acid copolymer.

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When a lactic acid/glycolic acid copolymer is used as the biodegradable polymer, its content ratio (lactic acid/glycolic acid) (mol%) is preferably about 100/0 to about 40/60, more preferably about 90/10 to about 50/50.

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The weight-average molecular weight of the lactic acid/glycolic acid copolymer is preferably about 5,000 to about 25,000, more preferably about 7,000 to about 20,000, still more preferably about 8,000 to about 15,000.

The degree of dispersion of the lactic acid/glycolic acid copolymer (weight-average molecular weight/number-average molecular weight) is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

The lactic acid-glycolic acid copolymer can be produced by a known production method, such as that described in Japanese Patent Unexamined Publication No. 28521/1986.

The decomposition/elimination rate of a lactic acid/glycolic acid copolymer varies widely, depending on composition or molecular weight. However, drug release duration can be extended by lowering the glycolic acid

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ratio or increasing the molecular weight, since decomposition/elimination is delayed as the glycolic acid ratio decreases. Conversely, drug release duration can be shortened by increasing the glycolic acid ratio or decreasing the molecular weight. To obtain a sustained-release preparation of the long acting type (e.g., 1-4 months), it is preferable to use a lactic acid-glycolic acid copolymer whose content ratio and weight-average molecular weight fall in the above ranges. If choosing a lactic acid-glycolic acid copolymer that decomposes more rapidly than that whose content ratio and weight-average molecular weight fall in the above ranges, the initial burst is difficult to suppress; if choosing a lactic acid-glycolic acid copolymer that decomposes more slowly than that whose content ratio and weight-average molecular weight fall in the above ranges, it is likely that no effective amount of drug is released for a certain period of time.

With respect to the formula [II] above, the linear or branched alkyl group represented by R, which has 2 to 8 carbon atoms, is exemplified by ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, tert-pentyl, 1-ethylpropyl, hexyl, isohexyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl and 2-ethylbutyl. Preferably, a linear or branched alkyl group having 2 to 5 carbon atoms is used. Such alkyl groups include ethyl, propyl, isopropyl, butyl and isobutyl. More preferably, R is ethyl.

The hydroxycarboxylic acid represented by the formula [II] is exemplified by 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxycaproic acid, 2-hydroxyisocaproic acid and 2-hydroxycapric acid, with preference given to 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methyl-

butyric acid and 2-hydroxycaproic acid, with greater preference given to 2-hydroxybutyric acid. Although the hydroxycarboxylic acid may be of the D-, L- or D,L-configuration, it is preferable to use a mixture of the D- and L-configurations wherein the ratio of the D-/L-configuration (mol%) preferably falls within the range from about 75/25 to about 25/75, more preferably from about 60/40 to about 40/60, and still more preferably from about 55/45 to about 45/55.

With respect to the copolymer of glycolic acid and the hydroxycarboxylic acid represented by the formula [II] (hereinafter glycolic acid copolymer (A)), polymerization may be of random, block or graft type. A random copolymer is preferred.

The hydroxycarboxylic acid represented by the formula [II] may be a mixture of one or more kinds in a given ratio.

With respect to the content ratio of glycolic acid and the hydroxycarboxylic acid represented by the formula [II] in glycolic acid copolymer (A), it is preferable that glycolic acid account for about 10 to 75 mol% and hydroxycarboxylic acid for the remaining portion. More preferably, glycolic acid accounts for about 20 to about 75 mol%, and still more preferably about 40 to about 70 mol%. The weight-average molecular weight of the glycolic acid copolymer is normally about 2,000 to about 50,000, preferably about 3,000 to about 40,000, and more preferably about 8,000 to about 30,000. The degree of dispersion of the glycolic acid copolymer (weight-average molecular weight/number-average molecular weight) is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

Glycolic acid copolymer (A) above can be produced by a known processes, such as that described in Japanese Patent Unexamined Publication No. 28521/1986.

Although the polylactic acid may also be of the D- or L-configuration or a mixture thereof, it is preferable that the ratio of the D-/L-configuration (mol%) falls within the range from about 75/25 to about 20/80. The ratio of the D-/L-configuration (mol%) is more preferably about 60/40 to about 25/75, and still more preferably about 55/45 to about 25/75. The weight-average molecular weight of the polylactic acid is preferably about 1,500 to about 30,000, more preferably about 2,000 to about 20,000, and still more preferably about 3,000 to about 15,000. Also, the degree of dispersion of the polylactic acid is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

For producing polylactic acid, two methods are known: ring-opening polymerization of lactide, a dimer of lactic acid, and dehydration polymerization condensation of lactic acid. For obtaining a polylactic acid of relatively low molecular weight for the present invention, direct dehydration polymerization condensation of lactic acid is preferred. This method is, for example, described in Japanese Patent Unexamined Publication No. 28521/1986.

Glycolic acid copolymer (A) and polylactic acid (B) are used in a mixture wherein the (A)/(B) ratio (% by weight) falls within the range from about 10/90 to about 90/10. The mixing ratio is preferably about 20/80 to about 80/20, and more preferably about 30/70 to about 70/30. If either component (A) or (B) is in excess, the preparation obtained shows a drug release pattern no more than that obtained with the use of component (A) or (B) alone; no linear release pattern is expected in the last stage of drug release from the mixed base. Although the decomposition/elimination rates of glycolic acid copolymer (A) and polylactic acid vary widely, depending on molecular weight or composition, drug release duration can be extended by increasing the molecular weight of the polylactic acid or lowering the mixing ratio (A)/(B), since the decomposi-

tion/elimination rate of glycolic acid copolymer (A) is usually higher than that of polylactic acid. Conversely, drug release duration can be shortened by decreasing the molecular weight of polylactic acid or increasing the mixing ratio (A)/(B). Drug release duration can also be adjusted by altering the kind and content ratio of hydroxycarboxylic acid represented by the formula [II].

In the present specification, weight-average molecular weight and degree of dispersion are defined as the molecular weight based on polystyrene obtained by gel permeation chromatography (GPC) with 9 polystyrenes as reference substances with respective weight-average molecular weights of 120,000, 52,000, 22,000, 9,200, 5,050, 2,950, 1,050, 580 and 162, and degree of dispersion calculated. Measurements were taken using a GPC column KF804Lx2 (produced by Showa Denko, Japan) and an RI monitor L-3300 (produced by Hitachi, Ltd., Japan) with chloroform as the mobile phase.

The production method of the present invention is hereinafter described in detail.

First, peptide [I] or a salt thereof (hereinafter also referred to as a drug) is dissolved or dispersed in water, with a drug support when necessary, such as gelatin, agar, polyvinyl alcohol or a basic amino acid (e.g., arginine, histidine, lysine), dissolved or suspended, to yield an internal aqueous phase.

The drug concentration in the internal aqueous phase is preferably about 0.1 to about 150% (w/v), more preferably about 20 to about 130% (w/v), and still more preferably about 60 to about 120% (w/v).

The internal aqueous phase also may be supplemented with a pH regulator for retaining drug stability and solubility, such as carbonic acid, acetic acid, oxalic acid, citric acid, phosphoric acid, hydrochloric acid, sodium hydroxide, arginine, lysine or salt thereof. In

addition, albumin, gelatin, citric acid, sodium ethylenediaminetetraacetate, dextrin, sodium hydrogen sulfite, polyol compounds such as polyethylene glycol, etc., as drug stabilizers, and p-oxybenzoates (e.g., methyl paraben, propyl paraben, etc.), benzyl alcohol, chlorobutanol, thimerosal etc., as preservatives, may be added.

The internal aqueous phase thus obtained is added to a solution containing a biodegradable polymer having a free carboxyl group at one end (hereinafter also referred to as polymer) (oil phase), followed by emulsification, to yield a W/O emulsion. This emulsification is achieved by a known dispersing method, such as the intermittent shaking method, the method using a mixer, such as a propeller stirrer or a turbine stirrer, the colloidal mill method, the homogenizer method or the ultrasonication method.

The above-described polymer-containing solution (oil phase) is prepared by dissolving a polymer in a substantially water-immiscible organic solvent. The water solubility of the organic solvent is preferably not higher than 3% (w/w) at normal temperature (20°C). Also, the boiling point of the organic solvent is preferably not higher than 120°C. Useful organic solvents include halogenated hydrocarbons (e.g., dichloromethane, chloroform, chloroethane, trichloroethane, carbon tetrachloride, etc.), alkyl ethers having 3 or more carbon atoms (e.g., isopropyl ether, etc.), alkyl ester (4 or more carbon atoms) of fatty acids (e.g., butyl acetate, etc.), aromatic hydrocarbons (e.g., benzene, toluene, xylene, etc.) and the like. These solvents may be used in combination. The organic solvent is more preferably a halogenated hydrocarbon (e.g., dichloromethane, chloroform, chloroethane, trichloroethane, carbon tetrachloride, etc.), and still more preferably dichloromethane.

The polymer concentration in the oil phase varies, depending on the molecular weight of the polymer and the kind of solvent, and is preferably about 0.01 to about 80% (w/w), more preferably about 0.1 to about 70% (w/w), and  
5 still more preferably about 1 to about 60% (w/w).

In a sustained-release preparation, the content ratio of drug varies depending on the kind of drug, desired pharmacologic effect, duration of action and other factors, and is about 0.01 to about 50% (w/w), relative to the base  
10 biodegradable polymer. The ratio is preferably about 0.1 to about 40% (w/w), more preferably about 1 to about 30% (w/w).

Next, the W/O emulsion thus produced is subjected to  
15 in-water drying. The in-water drying method is carried out by adding the W/O emulsion to an aqueous phase (external aqueous phase) to yield a W/O/W emulsion, and removing the solvent from the oil phase.

The volume of the external aqueous phase is normally  
20 selected within the range from about 1 to about 10,000 times, preferably about 2 to about 5,000 times, and more preferably about 5 to about 2,000 times, that of the oil phase.

An emulsifier may be added to the external aqueous  
25 phase. The emulsifier may be any one, as long as it is capable of forming a stable W/O/W emulsion. Such emulsifiers include anionic surfactants (e.g., sodium oleate, sodium stearate, sodium lauryl sulfate, etc.), nonionic surfactants [e.g., polyoxyethylene sorbitan fatty  
30 acid esters (Tween 80, Tween 60, Atlas Powder Company), polyoxyethylene castor oil derivatives (e.g., HCO-60, HCO-50, Nikko Chemicals), etc.], polyvinylpyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin, hyaluronic acid and the like. Among these, a  
35 preferred emulsifier is polyvinyl alcohol. These emulsifiers may be used singly or in combination. Their

concentration can be chosen as appropriate over the range from about 0.001 to about 20% (w/w), preferably from about 0.01 to about 10% (w/w), and more preferably from about 0.05 to about 5% (w/w).

5       An osmotic pressure adjustor may also be added to the above external aqueous phase.

Any osmotic pressure adjustor can be used in the invention, so long as it produces osmotic pressure in an aqueous solution thereof.

10       Examples of the osmotic pressure adjustor include water-soluble polyhydric alcohols; water-soluble monohydric alcohols; water-soluble monosaccharides, disaccharides and oligosaccharides or their derivatives; water-soluble amino acids; water-soluble peptides, proteins or their derivatives and the like.

15       Examples of the above water-soluble polyhydric alcohols include dihydric alcohols (e.g., glycerin, etc.), pentahydric alcohols (e.g., arabitol, xylitol, adonitol, etc.), hexahydric alcohols (e.g., mannitol, sorbitol, dulcitol, etc.) and the like. Among them, hexahydric alcohols, especially, mannitol is preferred.

20       Examples of the above water-soluble monohydric alcohols include methanol, ethanol, isopropyl alcohol and the like. Among them, ethanol is preferred.

25       Examples of the above water-soluble monosaccharides include pentoses (e.g., arabinose, xylose, ribose, 2-deoxyribose, etc.), hexoses (e.g., glucose, fructose, galactose, mannose, sorbose, rhamnose, fucose, etc.) and the like. Among them, hexoses are preferred.

30       Examples of the above water-soluble disaccharides include maltose, cellobiose,  $\alpha,\alpha$ -trehalose, lactose, sucrose and the like. Among them, lactose and sucrose are preferred.

35       Examples of the above water-soluble oligosaccharides include trisaccharides (e.g., maltotriose, raffinose,

etc.), tetrasaccharides (e.g., stachyose, etc.) and the like. Among them trisaccharides are preferred.

Examples of the derivatives of the above monosaccharides, disaccharides and oligosaccharides include  
5 glucosamine, galactosamine, glucuronic acid, galacturonic acid and the like.

Examples of the above water-soluble amino acids include neutral amino acids such as glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine,  
10 tryptophan, serine, threonine, proline, hydroxyproline, cysteine, methionine and the like; acidic amino acids such as aspartic acid, glutamic acid and the like; basic amino acids such as lysine, arginine, histidine and the like. There can also be used salts of these water-soluble amino  
15 acids with acids (e.g., hydrochloric acid, sulfuric acid, phosphoric acid, etc.) or alkalis (e.g., alkaline metals such as sodium, potassium and the like, etc.).

Examples of the water-soluble peptides, proteins or their derivatives include casein, globulin, prolamine,  
20 albumin, gelatin and the like.

Among these materials, water-soluble polyhydric alcohols; and water-soluble monosaccharides, disaccharides and oligosaccharides or their derivatives are preferred, water-soluble polyhydric alcohols and water-soluble  
25 monosaccharides being more preferred and water-soluble polyhydric alcohols being most preferred.

These osmotic pressure adjustors can be used alone or in combination thereof. A concentration of the osmotic pressure adjustor is selected so that the tonicity of the  
30 external aqueous phase is about 1/50 to about 5 times, preferably about 1/25 to about 3 times, that of physiological saline. For example, when the osmotic pressure adjustors are non-inonic materials, the concentration of these osmotic pressure adjustors in the  
35 external aqueous phase is about 0.001% to about 60% (w/w), preferably about 0.01 to about 40% (w/w), more preferably

about 0.05 to about 30% (w/w), particularly preferably about 1 to about 10% (w/w). When the osmotic pressure adjustors are ionic materials, they are used in a concentration calculated by dividing the above concentration by the total ionic valency. The osmotic pressure adjustors may be added so that their concentration becomes more than their solubility, and a part of them may be dispersed.

10 In the production method of the present invention, it is preferable that during formation of a W/O/W emulsion, the viscosity of the W/O emulsion be adjusted to about 150 cp to about 10,000 cp. Viscosity-adjusting methods include (1) adjusting the biodegradable polymer concentration of the oil phase, (2) adjusting the volume ratio of aqueous and oil phases, (3) adjusting the temperature of the W/O emulsion (4) adjusting external aqueous phase temperature, (5) and adjusting the temperature of the W/O emulsion using a line heater, cooler, or the like, during injection of the W/O emulsion to the external aqueous phase. These methods may be used singly or in combination.

20 In essence, in the present method it is necessary to adjust the viscosity of the W/O emulsion to about 150 cp to about 10,000 cp when the W/O emulsion turns to a W/O/W emulsion.

25 With respect to (1) above, the biodegradable polymer concentration in the oil phase cannot definitely be determined, because it varies depending on the kind of biodegradable polymer, kind of organic solvent and other factors, but is preferably about 10 to about 80% (w/w).

30 With respect to (2) above, the volume ratio of the aqueous and oil phases also cannot definitely be determined, because it varies depending on kind and amount of drug and oil phase nature, but the W/O ratio is preferably about 1 to about 50% (v/v).

35

With respect to (3) above, the temperature of the W/O emulsion, if adjusted, falls within the range from about -20°C to the organic solvent's boiling point, preferably about 0 to about 30°C, and more preferably about 10 to about 20°C.

The viscosity of the W/O emulsion viscosity can be adjusted during production of the W/O emulsion, in cases (1) and (2) above.

With respect to (4) above, it is recommended that the temperature of the external aqueous phase be previously adjusted before the W/O emulsion is added thereto, to yield results similar to those obtained in (3) above.

The temperature of the external aqueous phase is about 5 to about 30°C, preferably about 10 to about 25°C, and more preferably about 12 to about 20°C.

Organic solvent can be removed by known methods, including the method in which the solvent is evaporated under normal or gradually reduced pressure during stirring using a propeller stirrer, magnetic stirrer or the like, and the method in which the solvent is evaporated while the degree of vacuum is adjusted using a rotary evaporator or the like.

The thus-obtained sustained-release preparation, in the form of e.g., microcapsules ("microcapsules" may be also referred to as "microspheres"), is centrifuged or filtered to separate its particles, which are then washed with distilled water several times to remove the free drug, drug support, emulsifier etc. adhering to the microcapsules surface, and again dispersed in distilled water etc. and lyophilized.

An anticoagulant may be added to the above lyophilization. The anticoagulant is exemplified by water-soluble polysaccharides such as mannitol and starches (e.g., corn starch), inorganic salts, amino acids, and

proteins. The anticoagulant is preferably mannitol. The mixing ratio (weight ratio) of the microcapsules and anticoagulant is about 50:1 to about 1:1, preferably about 20:1 to about 1:1, still more preferably about 10:1 to about 5:1.

5

To prevent mutual aggregation of particles during washing, an anticoagulant may be added to the distilled water for washing. The anticoagulant is exemplified by water-soluble polysaccharides such as mannitol, lactose, glucose and starches (e.g., corn starch, etc.), proteins such as glycine, fibrin, collagen, etc., and inorganic salts such as sodium chloride, sodium hydrogen phosphate, etc. The preferred anticoagulant is mannitol.

10

After lyophilization, the microcapsules may be heated under reduced pressure to further remove the water and organic solvent therefrom, where desired.

15

If the heating temperature is below the glass transition temperature of the biodegradable polymer component, the effect of inhibiting the initial burst of the bioactive peptide will not be obtained. Conversely, if the temperature is too high, the risk of aggregation and deformation of microcapsules and decomposition or degradation of the bioactive peptide will be increased. The heating time cannot be specified in general terms but can be determined in consideration of the physical properties (e.g. molecular weight, stability, etc.) of the component biodegradable polymer, species of bioactive peptide, particle average diameter of microcapsules, heating time, degree of desiccation of microcapsules and heating procedure.

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As a preferred procedure, the microcapsules are heated at a temperature not below the glass transition temperature of the biodegradable polymer component and not so high as to cause aggregation of the microcapsules. For still better results, the heating temperature is preferably selected within the range from the glass transition

35

temperature of the biodegradable polymer component to about 30°C higher than the glass transition temperature of the component biodegradable polymer. Here, glass transition temperature is defined as the intermediate glass transition temperature determined using a differential scanning calorimeter during heating at a rate of 10 or 20°C per minute.

The heating time is also dependent on the heating temperature and the batch size of microcapsules, among other factors. Generally speaking, however, the heating time is preferably about 24 to about 120 hours, still more preferably about 48 to about 120 hours, after the microcapsules themselves have reached the specified temperature.

The heating method is not critical but any procedure conducive to a uniform heating of microcapsules can be employed.

As specific examples of such procedure, there may be mentioned heating in a constant-temperature bath, a fluidized bed, a moving bed or a kiln, and microwave heating. The most preferred method is heating in a constant-temperature bath.

By heating the microcapsules under reduced pressure after lyophilization, as stated above, the organic solvent is efficiently removed from the microcapsules, resulting in a biologically safe microcapsules. The residual organic solvent in the thus-obtained microcapsules is not more than about 100 ppm.

The thus-obtained microcapsules can be administered, as such or in the form of various dosage forms of non-oral preparations (e.g., intramuscular, subcutaneous or visceral injections or indwellable preparations, nasal, rectal or uterine transmucosal preparations, etc.) or oral preparations (e.g., capsules such as hard capsules and soft capsules, etc.), or solid preparations such as granules and

powders, or liquid preparations such as syrups, emulsions and suspensions.

5 An injectable preparation can be prepared by, for example, suspending microcapsules in water, along with a dispersing agent (e.g., Tween 80, HCO-60, carboxymethyl cellulose (including carboxymethyl cellulose sodium), sodium alginate, etc.), a preservative (e.g., methyl paraben, propyl paraben, etc.), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol, glucose, etc.) etc.,  
10 to yield an aqueous suspension, or by dispersing it in a vegetable oil such as sesame oil or corn oil, or the like, to yield an oily suspension, whereby a practically usable sustained-release preparation is obtained.

15 When microcapsules are used as an injectable suspension, for instance, their average particle size is chosen over the range from about 0.1 to about 500  $\mu\text{m}$ , as long as the requirements concerning degree of dispersion and needle passage are met. Preferably, the average particle size is about 1 to about 300  $\mu\text{m}$ , and more  
20 preferably about 2 to about 200  $\mu\text{m}$ .

When the sustained-release preparation is microcapsules, by adding the osmotic pressure adjustor as mentioned above, its particle shape become better spheric shape which is better for needle pasage.

25 Methods of preparing microcapsules as a sterile preparation include, but are not limited to, the method in which the entire production process is sterile, the method in which gamma rays are used as sterilant, and the method in which an antiseptic is added.

30 The sustained-release preparation of the present invention is not significantly toxic and can be used safely in mammals (e.g., humans, bovines, swines, dogs, cats, mice, rats, rabbits, etc.).

35 Although varying widely depending on kind, content and dosage form, and duration of release of the drug, target

disease (e.g., hormone-dependent diseases such as prostatic cancer, prostatic hypertrophy, endometriosis, uterine myoma, precocious puberty, breast cancer, bladder cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, gastritis, 5 hodgekin's disease, malignant melanoma, metastasis, multiple myeloma, non-hodgekin's leukemia, non-small-cell lung cancer, ovarian cancer, peptic ulcer, serious fungal infection, small-cell lung cancer, valvular heart disease, 10 mastopathy, polycystic ovary, infertility, controlled induction of ovulation in women with chronic anovulation, acne, amenorrhea (e.g., secondary amenorrhea), ovarian and mammary cystic disease (including polycystic ovarian diseasea), gynecological cancer, ovarian hyperandrogenism and hirsutism, AIDS by rejuvenating the thymus to produce 15 T-cells, male contraceptives for treatment of male sex offenders and in contraception, symptomatic relief of the premenstrual syndrome (PMS), in vitro fertilization), subject animal species and other factors, the dose of the sustained-release preparation may be set at any level, as 20 long as the desired effect of the drug is obtained. The dose of the drug per administration can be chosen as appropriate over the range from about 0.01 mg to about 100 mg/kg body weight, preferably from about 0.05 mg to about 25 50 mg/kg body weight, and more preferably from about 0.1 mg to about 10 mg/kg body weight per adult, in the case of a 1-month release preparation.

The dose of the sustained-release preparation per administration can be chosen as appropriate within the 30 range from about 0.1 mg to about 500 mg/kg body weight, preferably from about 0.2 mg to about 300 mg/kg body weight per adult. The frequency of administration can be chosen as appropriate, depending on kind, content and dosage form, duration of release of the drug, target disease, subject 35 animal species and other factors, e.g., once every several weeks, once every month or once every several months.

Best Mode for Carrying out the Invention

The present invention is hereinafter described in more detail by means of the following reference examples and working examples, which are not to be construed as  
5       limitative. In the examples below, % values are by weight, unless otherwise stated.

Example 1

10       500 mg of the acetate (produced by TAP Company) of N-(S)-2-tetrahydrofuroyl-Gly-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH<sub>2</sub> (hereinafter referred to as peptide A) was dissolved in 0.6 ml of distilled  
15       water. The resulting solution was added to a solution of 4.5 g of a lactic acid-glycolic acid copolymer (hereinafter referred to as PLGA) [produced by Wako Pure Chemical, Japan, lot. 940810; lactic acid/glycolic acid (molar ratio), 74/26, weight-average molecular weight based on  
20       GPC, 10,000; number-average molecular weight based on GPC, 3,900; number-average molecular weight based on terminal group quantitation, 3,700] in 5.8 ml of dichloromethane, followed by homogenization for 60 seconds in a small homogenizer (produced by Kinematica Company) to yield a W/O emulsion. After being cooled to 16°C, the W/O emulsion was  
25       poured over 1,000 ml of a 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry Co., Ltd.), previously adjusted to 16°C, and then prepared as a W/O/W emulsion using a turbine type homomixer (produced by Tokushu Kika) at 7,000 rpm. This  
30       W/O/W emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and solidify the W/O emulsion, which was then centrifuged at 2,000 rpm using a centrifuge (05PR-22, Hitachi Limited). The resulting precipitate was again dispersed in distilled water,  
35       followed by centrifugation and washing down of the free drug. After the collected microcapsules were again

dispersed in a small amount of distilled water, 0.3 g of D-mannitol was added to the dispersion, which was lyophilized to yield powdered microcapsules. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu\text{m}$  and 9.5% (w/w), respectively.

#### Example 2

Microcapsules were obtained in the same manner as in Example 1, except that PLGA [produced by Wako Pure Chemical, lot. 940813; lactic acid/glycolic acid (molar ratio), 73/27; weight-average molecular weight based on GPC, 13,000; number-average molecular weight based on GPC, 4,500; number-average molecular weight based on terminal group quantitation, 4,700] was used. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu\text{m}$  and 9.5% (w/w), respectively.

#### Example 3

Microcapsules were obtained in the same manner as in Example 1, except that PLGA [produced by Wako Pure Chemical, lot. 940808; lactic acid/glycolic acid (molar ratio), 74/26; weight-average molecular weight based on GPC, 7,800; number-average molecular weight based on GPC, 3,500; number-average molecular weight based on terminal group quantitation, 3,000) was used. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu\text{m}$  and 9.5% (w/w), respectively.

#### Example 4

Microcapsules were obtained in the same manner as in Example 1, except that the amount of peptide A acetate was 794 mg. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu\text{m}$  and 14.3% (w/w), respectively.

#### Example 5

15 g of peptide A acetate was dissolved in 18 ml of distilled water. The resulting solution was added to a solution of 135 g of PLGA [produced by Wako Pure Chemical, lot. 940810; lactic acid/glycolic acid (molar ratio), 74/26; weight-average molecular weight based on GPC, 10,000; number-average molecular weight based on GPC, 3,900; number-average molecular weight based on terminal group quantitation, 3,700) in 174 ml of dichloromethane, followed by homogenization in a homogenizer to yield a W/O emulsion. This W/O emulsion was poured over 30 l of a 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry Co., Ltd.), previously adjusted to 17°C, and was then prepared as a W/O/W emulsion using an in-line type homomixer. This W/O/W emulsion was stirred at room temperature to volatilize off the dichloromethane and solidify the W/O emulsion, which was then centrifuged. The resulting precipitate was washed with distilled water to remove the free drug. After the collected microcapsules were again dispersed in a small amount of distilled water, 13.5 g of D-mannitol was added to the dispersion, which was lyophilized and then dried under reduced pressure in a constant-temperature chamber at 40-43°C for 19 hours, then at 42-44°C for 48 hours to yield powdered microcapsules. The particle size distribution and peptide A content of the microcapsules were 3-60  $\mu\text{m}$  and 8.7% (w/w), respectively.

#### Example 6

Microcapsules were obtained in the same manner as in Example 1, except that the acetate of NAcD2Nal-D4ClPhe-D3Pal-Ser-Tyr-DhArg(Et<sub>2</sub>)-Leu-hArg(Et<sub>2</sub>)-Pro-DAlaNH<sub>2</sub> (produced by Syntex Company) was used in place of peptide A acetate. The particle size distribution and peptide content of the microcapsules were 5-60  $\mu\text{m}$  and 9.4% (w/w), respectively.

Example 7

857 mg of peptide A acetate was dissolved in 0.8 ml of distilled water. The resulting solution was added to a solution of 4.5 g of PLGA [produced by Wako Pure Chemical, lot. 950526; lactic acid/glycolic acid (molar ratio), 74/26; weight-average molecular weight based on GPC, 11,700; number-average molecular weight based on GPC, 5,200; number-average molecular weight based on terminal group quantitation, 3,800) in 6 ml of dichloromethane, followed by homogenization in a homogenizer to yield a W/O emulsion. Microcapsules were obtained in the same manner as in Example 1, except that 0.5 g of D-mannitol was added to the dispersion, which was lyophilized to yield powdered microcapsules. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu$ m and 11.7% (w/w), respectively

Example 8

Microcapsules were obtained in the same manner as in Example 1, except that the amount of peptide A acetate was 1125 mg, the amount of distilled water was 1.0 ml, the amount of dichloromethane was 6.3 ml. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu$ m and 11.7% (w/w), respectively.

Example 9

Microcapsules were obtained in the same manner as in Example 7, except that the amount of peptide A acetate was 1421 mg, the amount of distilled water was 1.2 ml, the amount of dichloromethane was 6.7 ml. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu$ m and 17.5% (w/w), respectively.

Example 10

Microcapsules were obtained in the same manner as in Example 8, except that 50 g of D-mannitol was added to

1,000 ml of the 0.1% aqueous solution of polyvinyl alcohol. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu\text{m}$  and 14.7% (w/w), respectively.

5     Example 11

Microcapsules were obtained in the same manner as in Example 9, except that 50 g of D-mannitol was added to 1,000 ml of the 0.1% aqueous solution of polyvinyl alcohol. The particle size distribution and peptide A content of the  
10     microcapsules were 5-60  $\mu\text{m}$  and 17.0% (w/w), respectively.

Reference Example 1

1125 mg of peptide A and 4.5 g of PLGA [produced by Wako Pure Chemical, lot. 950526; lactic acid/glycolic acid (molar ratio), 74/26, weight-average molecular weight based  
15     on GPC, 11,700; number-average molecular weight based on GPC, 5,200; number-average molecular weight based on terminal group quantitation, 3,800] were dissolved in 6.0 ml of dichloromethane. After being cooled to 16°C, the  
20     solution was poured over 1,000 ml of a 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry Co., Ltd.), previously adjusted to 16°C, and then prepared as a O/W emulsion using a turbine type homomixer (produced by Tokushu Kika) at  
25     7,000 rpm. This O/W emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane, which was then centrifuged at 2,000 rpm using a centrifuge (05PR-22, Hitachi Limited). The resulting precipitate was again dispersed in distilled  
30     water, followed by centrifugation and washing down of the free drug. After the collected microcapsules were again dispersed in a small amount of distilled water, 0.5 g of D-mannitol was added to the dispersion, which was lyophilized to yield powdered microcapsules. The particle size  
35     distribution and peptide A content of the microcapsules were 5-60  $\mu\text{m}$  and 13.2% (w/w), respectively.

Reference Example 2

Microcapsules were obtained in the same manner as in Reference Example 1, except that the amount of peptide A acetate was 1421 mg, the amount of dichloromethane was 6.2 ml. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu$ m and 15.9% (w/w), respectively.

Reference Example 3

Microcapsules were obtained in the same manner as in Reference Example 2, except that 50 g of D-mannitol was added to 1,000 ml of the 0.1% aqueous solution of polyvinyl alcohol. The particle size distribution and peptide A content of the microcapsules were 5-60  $\mu$ m and 15.5% (w/w), respectively.

Experimental Example 1

About 20 mg of the microcapsules obtained in Example 4 was dispersed in 0.5 ml of dispersing solvent (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol dissolved therein), and injected subcutaneously to the backs of male SD rats at 10 weeks of age, using a 22-G injection needle. After administration, rats were sacrificed at constant intervals; the remaining microcapsules were taken out from the injection site; microcapsules peptide A quantitation results are shown in Table 1.

Table 1

Time after Administration	Ratio of Residual Peptide A (%)
1 day	96.4
1 week	84.8
2 weeks	59.2
3 weeks	38.8
4 weeks	24.6

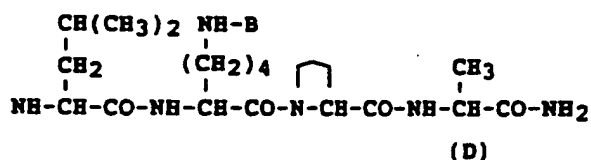
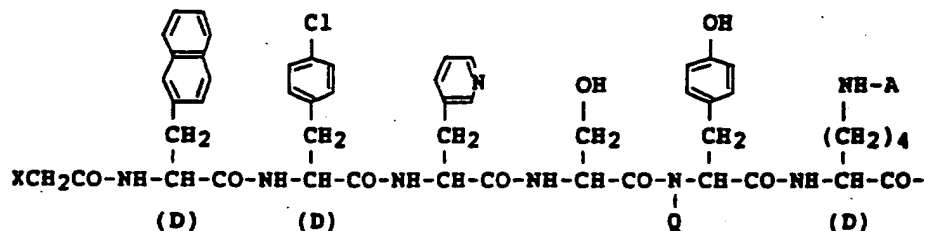
As shown in Table 1, the microcapsules obtained according to the production method of the present invention release peptide A constantly, with substantially no initial burst.

#### Industrial Applicability

According to the present Invention, a sustained-release preparation containing peptide [I] or a salt thereof can be obtained easily and at high recover rates.

## CLAIMS

1. A method of producing a sustained-release preparation, which comprises producing a W/O emulsion whose internal aqueous phase is a solution containing a bioactive peptide represented by the formula:



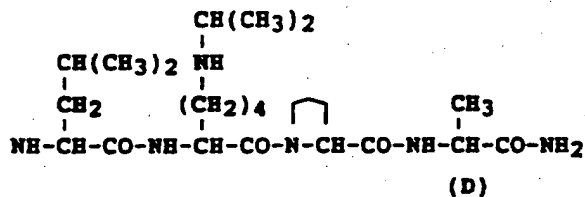
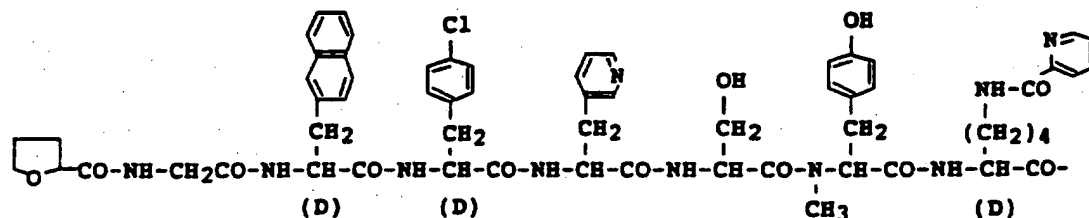
wherein X represents a hydrogen atom or a tetrahydrofuryl-carboxamido; Q represents a hydrogen atom or methyl; A represents nicotinoyl or N,N'-diethylamidino; B represents isopropyl or N,N'-diethylamidino, or a salt thereof, and whose oil phase is a solution containing a biodegradable polymer having a free carboxyl group at one end, and adding the W/O emulsion into an external water phase to produce W/O/W emulsion.

2. A method of claim 1, wherein the biodegradable polymer is an aliphatic polyester.

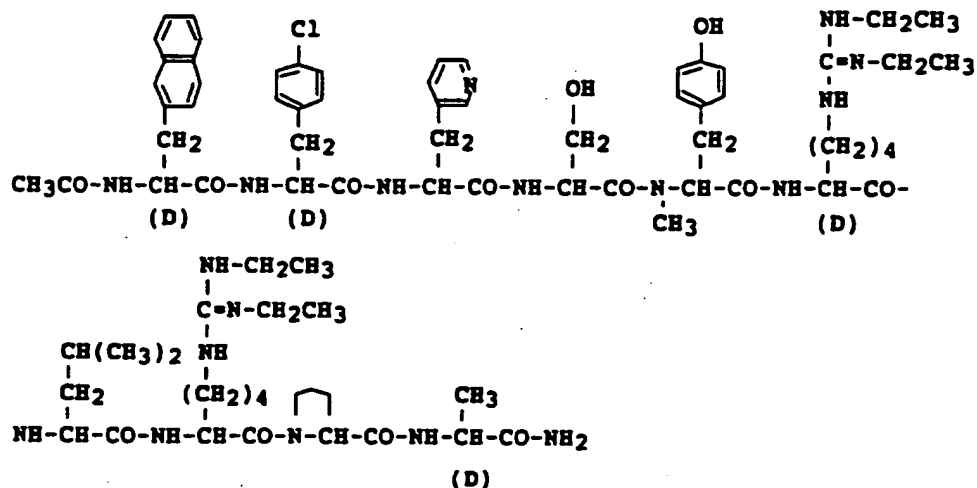
3. A method of claim 2, wherein the aliphatic polyester is a lactic acid-glycolic acid copolymer.

4. A method of claim 3, wherein a composition ratio of lactic acid and glycolic acid is about 100/0 to about 40/60 (mole%).

5. A method of claim 3, wherein a weight-average molecular weight of the copolymer is about 5,000 to about 25,000.



14. A method of claim 1, wherein the peptide is of the formula:



15. A sustained-release preparation, which is produced by the method of claim 1.
16. A preparation of claim 15, wherein a content ratio of the peptide is about 0.01 to about 50% (w/w), relative to the polymer.
17. A preparation of claim 15, wherein the preparation is microcapsules.
18. A preparation of claim 17, wherein the microcapsules are for injection.

## INTERNATIONAL SEARCH REPORT

International Application No

PLI/JP 96/00090

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K38/09 A61K9/16 A61K9/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 601 799 (TAKEDA CHEMICAL INDUSTRIES LTD.,JP) 15 June 1994 cited in the application see claims see page 11, line 17 - line 46 ---	1-18
A	EP,A,0 413 209 (ABBOTT LABORATORIES,U.S.A.) 20 February 1991 cited in the application see claims see page 20, line 14 - line 49 -----	1-18

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

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Date of the actual completion of the international search

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Scarponi, U

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PLI/JP 96/00090

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		CA-A- 2110730	08-06-94
		FI-A- 935471	08-06-94
		WO-A- 9413317	23-06-94
		JP-A- 7097334	11-04-95
		NO-A- 934423	08-06-94
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		US-A- 5300492	05-04-94

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3

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